Protocol for mini-prep

with GeneMark Plasmid Miniprep Purification Kit

1. Pellet 1~10 ml of bacteria culture by centrifugation for 1 min at top speed (12~14,000x g) in a microcentrifuge. Discard the supernatant and remove any excess media.
Note: For liquid culture > 5 ml, increase Solution I, Solution II and Solution III volume to prevent product loss.

2. Resuspend the cell pellet completely in 200 µl of Solution I by pipetting or vortexing.

3. Add 200 µl of Solution II and mix by inverting the tube 5 times; the cell suspension should turn clear immediately.

4. Add 200 µl of Solution III and mix by inverting the tube 5 times.

5. Centrifuge the lysate at top speed in a microcentrifuge for 5 min. A compact white pellet will form along the side or at the bottom of the tube.

6. Insert the Spin Column into a Collection Tube, carefully transfer all of the clear lysate from step 5 to spin column, centrifuge at top speed for 1 min.

7. Discard the filtrate from the collection tube and add 700 µl of Wash Solution to the spin column and centrifuge at top speed for 1 min. Repeat this step once more.

8. Discard the filtrate and centrifuge at top speed for additional 3~5 min to remove residual trace of ethanol.
* If centrifugation speed is lower than 10,000x g or residual ethanol must be removed completely, incubate the spin column in a heat oven (45~60°C) for 5 min to evaporate all of the ethanol.

9. Transfer the spin column into a new microcentrifuge tube and add 50~100 µl of Elution Solution or H_2O (pH 7.0~8.5) into the column and wait for 1~2 min. (For plasmid DNA larger than 7 kb, use preheated (60~70°C) Elution solution to elute.)

10. Centrifuge at top speed for 1 min to elute the DNA. Store the eluted plasmid DNA at -20°C.
*The yield of plasmid DNA is 6~60 µg for 1~10 ml E. coli culture at purity of 1.8~2.0 (A260/A280).

Reference: GeneMark® Plasmid Miniprep Purification Handbook